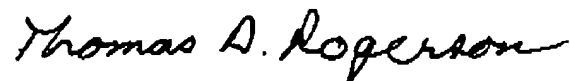


suggestion that orchids are treated with 1-methylcyclopropene at reduced pressure. What the abstract does teach is that fumigation of the orchids with insecticide is conducted at low pressure and that adverse effects of the fumigation is alleviated by "pretreatment" with 1-MCP (abstract translation, lines 13-19).

Page 4 of the translation, section number 2 discloses that the orchid flowers are first treated with 1-methylcyclopropene under ambient conditions and then fumigated at reduced pressure. (see translation page 4, lines 7-13). In most of the examples there is a simulated transportation step between the 1-MCP treatment and fumigation. There is no disclosure, teaching, or suggestion in Huang alone, or in combination with Sisler, that plants are, or should be, treated with 1-MCP at reduced pressures. Thus, the reference does not disclose Applicants' claimed invention; the treatment of plants and plant parts with 1-methylcyclopropene at reduced pressure and the advantages of such treatment. Unfortunately, this is a case where the abstract of the paper did not adequately describe what actually occurred in the experiments described in the paper or the results and conclusions of the paper. One of ordinary skill in the art who was familiar with both Sisler and Huang would not be motivated to use the low-pressure technique of fumigation for the treatment of plants with 1-MCP based upon the teachings of these references.

With this response, Applicants believe that the rejections have been overcome and the claims are in condition for allowance. Should the Examiner have any suggestions which may put the Application in better condition for allowance, Applicants' attorney is willing to discuss any such suggestions either by phone or at the U. S. Patent and Trademark Office.

Respectfully submitted,



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## TRANSLATION FROM CHINESE

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## **Preservative Effect of 'TARI's Onc 1' and 1-MCP on Oncidium Cut Flowers Following Simulated Transport to Japan and Quarantine Fumigation**

Key words: Oncidium, pretreatment, quarantine, fumigation, simulated transportation

**Abstract:** Exports of Oncidium cut flowers have increased rapidly in recent years, with the bulk of these exports going to Japan, and this flower already accounts for the second largest export volume among flowers in Taiwan. However, some 20-30% of these flowers are fumigated in Japan because of plant pests (chiefly moth larvae). In the present study, Oncidium cut flowers were fumigated with methyl bromide following simulated transport to Japan ( $24.5 \text{ g/m}^3$ , the dose used in Japan), and it was found that although this fumigation had little effect on the auction quality of the cut flowers, it did reduce their vase life by 1-2 days. By treating Oncidium cut flowers prior to transport with 'TARI's Onc 1' and 1-MCP (1-methylcyclopropene), it was possible to increase the vase life of cut flowers after fumigation by 1-2 days, simultaneously improving their vase quality to a level close to that of unfumigated flowers. Methyl bromide fumigation also decreases bud opening and increases yellowing rates, and pretreatment with 'TARI's Onc 1' and 1-MCP can effectively alleviate this and return these two rates close to the levels seen in unfumigated flowers. It was found that when flowers are treated prior to transport by the low-pressure fumigation method developed by the Bureau of Animal and Plant Health Inspection and Quarantine (e.g., fumigation for three hours at low pressure (250 torr) with a mixture of methyl bromide ( $10 \text{ g/m}^3$ ) and phosphine ( $3 \text{ g/m}^3$ ), adverse effects on quality and vase life following transport of Oncidium cut flowers were minor compared to those seen in high-dose fumigation with methyl bromide ( $24.5 \text{ g/m}^3$ ), and these adverse effects could be effectively alleviated by pretreatment with 'TARI's Onc 1' and 1-MCP. Mortality rates of close to 100%

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were seen for *Spodoptera litura*, *Aphis gossypii*, and *Thrips hawaiiensis*. Low-pressure fumigation with phosphine alone ( $5 \text{ g/m}^3$ ) yielded a 100% mortality rate for *Spodoptera litura*, but a small number of the pests *Aphis gossypii* and *Thrips hawaiiensis* (1.7-11.6%) were found to have survived immediately after fumigation; following simulated air transport, the survival rates for *Aphis gossypii* and *Thrips hawaiiensis* dropped to 5.3% and 0.0%, with rates following simulated ocean transport of 2.0% and 0.3% respectively. The results clearly demonstrated that appropriate preservative treatment can alleviate damage caused by fumigation of *Oncidium* cut flowers after export to Japan, and that low-pressure fumigation shows a favorable effect on insect mortality, and it is worth conducting further research on practical methods of appropriate preservative treatment in order to further ensure cut flower quality.

### Introduction

Exports of *Oncidium* spp. cut flowers from Taiwan to Japan reached a level of more than 9,000,000 stems in 1999, with a figure of over 1.2 million stems in 2000, and this flower now accounts for the second highest export volume among flowers in Taiwan after the chrysanthemum. As export volume has increased, there has also been increasing fumigation of these flowers in Japan, particularly in the spring. The vase life of *Oncidium* after export already tends to be somewhat short, and it is further shortened by fumigation, causing a decrease in the sales price. Therefore, the development of techniques for protecting flowers from the effects of fumigation would make it possible to restore cut flower quality to normal following fumigation in Japan and would be of great benefit to the industry. If further research can be conducted on favorable insecticidal technology, this will make it possible to effectively eradicate pests prior to export without damaging the flowers, not only reducing the high cost of fumigation in Japan but resulting in more rapid customs clearance and preventing losses due to delayed sales.

There has been little research conducted abroad on methods for protecting *Oncidium* cut flowers, but it was reported in Singapore that harvesting flowers at an earlier stage reduces crushing damage during export, and that treatment with a mixture of 8HQS (8-hydroxyquinoline sulfate) or acetylsalicylic acid mixed with sucrose can increase bud opening rates and prolong cut flower life span (Hew, 1987). When ethylene is used in treating *Oncidium* cut flowers, the flowers do not show accelerated senescence, indicating that *Oncidium* is not sensitive to ethylene (Goh et al., 1985).

In order to cope with increasing exports of cut flowers to Japan in recent years, investigations have been conducted in this country on methods for preserving freshness during transport of

other orchid varieties, such as studies on preservation of freshness during air transport in the butterfly orchid (Huang et al., 1988) and studies on data concerning the physiology and preservation of vase freshness of butterfly orchids (Lin and Li, 1988, Yang et al., 1992). These studies have shown that preservation of freshness during transport of *Oncidium* cut flowers differs from that of butterfly orchid cut flowers. However, it is known that *Oncidium* cut flowers have a life span after transport to Japan of approximately 5-7 days, and this life span is reduced by half by fumigation (Huang, 1998a). The rapid senescence of *Oncidium* cut flowers is associated with loss of the anther covering, with low intrinsic nutrient levels causing a shorter life span, and added ethylene clearly accelerates flower senescence and bud yellowing (Huang, 1998b). In order to counter these problems, the Taiwan Agricultural Research Institute developed a special method for preserving freshness in *Oncidium* cut flowers (Huang, 2000) involving treatment with 'TARI's Onc 1' and 1-MCP (1-methylcyclopropene) (see Section 2 under Materials and Methods for information on this treatment method). In order to improve the quality of *Oncidium* cut flowers exported to Japan, it is also necessary to deal with the issue of quarantine treatment after export. In addition to researching methods for reducing the amount of fumigation flowers undergo, it is also necessary to study technologies for preserving freshness in order to prevent fumigation after reaching Japan from having too great an effect on the vase quality and life span of *Oncidium* cut flowers. In this study, we also investigated the effect on *Oncidium* cut flowers of an insecticidal treatment method for chrysanthemums newly developed by the Taichung Office of the Bureau of Animal and Plant Health Inspection and Quarantine (Chen et al., 1993, Zhang et al., 1994). It is hoped that this method can be used to successfully eradicate pests while reducing damage to *Oncidium* cut flowers after transport in order to improve their quality after export to Japan.

## Materials and Methods

### 1. Cut flowers

The variety of *Oncidium* cut flowers used in the study was *Onc. Gower Ramsey (Onc. Goldiana x Onc. Guiena Gold)* 'Nanxi.' The flowers, which were obtained from Dalin Chiayi Orchid Production and Sales Group, were approximately 70% mature when harvested and were of export standard grade B. The flowers were 45 cm or more in length, the stems were approximately 40 cm in length, each having 5-7 branches and 4-5 small buds, and the plants were free of disease and insect pests. The flowers were immediately placed in water after being harvested. After being sent back to the laboratory, the anthers were removed from the open

flowers, and the buds and flowers were labeled and separated. After screening for uniform external appearance and quality, they were re-cut to a stem length of approximately 35 cm, tied in bunches of 10, and covered with a protective sleeve.

## 2. Cut flower treatment and quality measurement

The cut flowers were divided into two groups, i.e. a control group and a group treated with 'TARI's Onc 1' and 1-MCP (1-methylcyclopropene). According to the current method, the flowers in the control group were preserved with tap water and placed in a protective tube. In the group treated with 'TARI's Onc 1' and 1-MCP, the cut flowers were first preserved with 'TARI's Onc 1,' then treated for 4 hours at room temperature ( $25 \pm 1^\circ\text{C}$ ) with MCP  $1.0 \text{ g/m}^3$  and placed in a preservative tube containing 'TARI's Onc 1' as a freshness preserving agent. Following simulated transport in a container, the flowers were fumigated with methyl bromide (with those undergoing low-pressure fumigation being fumigated prior to transport), after which auction quality, vase life, and deterioration of quality were investigated.

Auction quality was evaluated after the cut flowers had undergone simulated air or ocean transport by allowing them to stand at room temperature for one day, removing them from the box on the following day, and carrying out pre-scoring based on the degree of cracking of the lip of the flower and bud yellowing, with flowers showing quality equivalent to that of the original harvested flowers being given a score of 2. The more extensive cracking was and the greater the severity of bud yellowing, the lower the score, with a score of 0 indicating a product of no value. Vase life was determined based on the flowers and buds on the branches of the main stem, with the quality of each flower and bud being recorded daily. Flowers showing pronounced senescence (clear cracking of the lip and curling) and buds showing severe yellowing were considered to be dead, with the day on which 1/2 of the flowers and buds on each main stem were dead being taken as the end of that flower's vase life.

## 3. Methyl bromide fumigation equipment

Methyl bromide fumigation was conducted outdoors in a small temperature-controlled tent made of highly airtight plastic fabric measuring [illegible] cm, 50 cm, and 148 cm in length, width, and height respectively (volume  $0.46 \text{ m}^3$ ), and its airtightness was measured and confirmed to be favorable (increase in 24-hour oxygen concentration from 0% to 0.2%). This fumigation tent and the small refrigerator used were equipped with air exhaust equipment, thus keeping the effect of gas leakage from the two chambers on humans to a minimum. Methyl

bromide was introduced via a plastic tube, with the amount [illegible] being measured with an electronic scale and artificially controlled.

#### 4. Effect of methyl bromide fumigation after transport

##### Effect of methyl bromide fumigation treatment following simulated air transport on quality of Oncidium cut flowers

After general treatment and treatment with 'TARI's Onc 1' and 1-MCP, the Oncidium cut flowers were placed in boxes and allowed to stand for 3 or 4 days at room temperature ( $25 \pm 1^\circ\text{C}$ ) in order to simulate air transport to Japan, after which a portion of the cut flowers was placed in the fumigation tent and methyl bromide fumigation with  $24.5 \text{ g/m}^3$  of methyl bromide was conducted for 2 hours while maintaining the temperature at  $23^\circ\text{C}$  (the fumigation conditions used in Japan). After this, the tent was vented overnight, and on the next day, the flowers were evaluated for auction quality together with the unfumigated group, with 6 flowers in each treatment group being selected and [illegible] 6 times. After being cut, the flowers were placed in a vase in a laboratory at room temperature of  $26 \pm 2^\circ\text{C}$  and relative humidity of  $70 \pm 15\%$ , and their vase life was measured.

##### Effect of methyl bromide fumigation following simulated ocean transport on quality of Oncidium cut flowers

Following general treatment and 'TARI's Onc 1' treatment of the cut flowers, the boxes were placed in a refrigerator at  $12.0 \pm 0.5^\circ\text{C}$  for 7 days or  $11.0 \pm 0.5^\circ\text{C}$  for 8 days in order to simulate conditions during ocean transport to Japan. After this, the flowers were fumigated with methyl bromide (Section 4(1)) and their quality was measured ((2)).

##### Effect of low-pressure fumigation prior to transport on quality of Oncidium cut flowers following simulated transport and effect on insect mortality

After being placed in containers, the Oncidium cut flowers were first subjected to low-pressure fumigation treatment to eradicate pests, after which they were subjected to simulated air and ocean transport, and their quality and vase life and the effect on insect mortality were then evaluated. Low-pressure fumigation was carried out according to the method developed by the Taichung Office of the Bureau of Animal and Plant Health Inspection and Quarantine, with this method involving (1) fumigation with  $10 \text{ g/m}^3$  of methyl bromide and  $3 \text{ g/m}^3$  of phosphine (aluminum phosphide), and (2) fumigation with  $5 \text{ g/m}^3$  of phosphine (aluminum phosphide) alone, with both of these processes being carried out at the low pressure of 250 torr. Low-pressure fumigation was carried out at the Taichung Office of the Bureau of Animal and Plant

Health Inspection and Quarantine, with fumigation lasting 3 hours each time, and the total time required for addition of the chemicals and venting was 4 [illegible] hours.

Three plant pests were used in the study, with all three of these species being commonly seen on cut flowers. Among these, *Thrips hawaiiensis* and *Aphis gossypii* were taken from the field and placed on chrysanthemums, the chrysanthemum stems were cut short, the flowers were placed in a tube to preserve freshness, the entire flower was covered with a non-woven fabric in order to prevent the pests from escaping, and they were then placed in the *Oncidium* paper box [illegible] for fumigation. *Spodoptera litura* (2- and 3-year) were provided by the Department of Entomology, National Chung Hsing University they were placed in a small PVC box with a ventilation [illegible] net and placed in the paper box, with each box containing two portions. After low-pressure fumigation, one sample was taken from each of the boxes and tested for mortality. While [illegible] in the paper boxes, the mortality rates of the pests were again tested following simulated air and ocean transport of *Oncidium*.

## Results

### Effect of methyl bromide fumigation after transport on auction quality and vase life of *Oncidium* cut flowers

Methyl bromide fumigation following transport caused a slight decrease in the auction quality and flower and bud quality of *Oncidium* cut flowers following simulated air and ocean transport (Table 1), and it also caused decreases in the vase life of the cut flowers, with the decreases for air and ocean transport respectively being 0.9 and 1.4 days. Flower life span, bud life span, and bud opening rates also decreased (Table 1).

Methyl bromide fumigation following transport increased bud yellowing rates of the cut flowers in the vase (Table 2), but had little effect on flower diameter.

### Effect of treatment with 'TARI's Onc 1' and 1-MCP (1-methylcyclopropene) on quality of *Oncidium* cut flowers subjected to methyl bromide fumigation following transport

Treatment with 'TARI's Onc 1' and 1-MCP (1-methylcyclopropene) increased the auction quality of the *Oncidium* cut flowers following simulated air and ocean transport (Table 1), and it also prolonged vase life by 2-3 days and increased flower life span, bud life span, and bud opening rates. Treatment of *Oncidium* cut flowers that had already undergone methyl bromide fumigation with 'TARI's Onc 1' and 1-MCP also increased vase life span, with increases of 1.9 days and [illegible] 3 days following air and ocean transport respectively, making the life span of the cut flowers longer than in the air transport control group and similar to that of the ocean

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transport control group. Treatment with 'TARI's Onc 1' and 1-MCP also reduced bud yellowing caused by fumigation (Table 2) causing a decrease in yellowing rates. In addition, treatment with 'TARI's Onc 1' and 1-MCP increased Oncidium flower diameter, thus improving the aesthetic appearance of the flowers.

Table 1. Effect of 'TARI's Onc 1' and 1-MCP (methylcyclopropene) treatment on auction quality, flower and bud senescence, vase life, flower and bud life span, and bud opening rates of Oncidium cut flowers following simulated transport to Japan and methyl bromide fumigation

Simulated transportation	Treatment		Auction			Vase life (day)			Bud opening (%)
	TARI's Onc1+MCP	MBr Fumi.	Quality (-2~+2)	Floret sene.	Bud sene.	Cut flower	Floret	Bud	
25°C 4 days	-	-	1.3±0.2	slight	none	63±1.0	48±1.1	98±2.6	61.7±12.2
	-	+	1.5±0.3	slight	none	54±1.2	42±0.4	81±1.6	47.5±8.7
	+	-	1.6±0.3	none	none	80±1.8	58±1.1	122±1.6	64.5±19.7
	+	+	1.7±0.4	none	none	75±1.8	63±1.2	120±1.7	58.8±13.2
12°C 7 days	-	-	1.5±0.4	slight	none	61±1.4	54±0.6	82±2.4	45.3±12.4
	-	+	1.4±0.6	slight	none	47±0.5	38±1.0	62±2.0	28.8±5.4
	+	-	1.8±0.5	none	none	93±0.8	64±0.6	128±0.8	76.3±16.2
	+	+	1.7±0.5	none	none	60±2.0	54±0.8	82±3.2	45.8±12.8

Notes: 1. fumi. = fumigation for 2 hours with 24.5 g/m<sup>3</sup> of methyl bromide at 23 °C following simulated transport.

2. Quality = indicates auction quality: +2: Extremely good, +1: Good, 0: Neither good nor bad, -1: Poor, -2: Extremely poor.

3. sene. = senescence.

Table 2. Effect of 'TARI's Onc 1' and 1-MCP (methylcyclopropene) treatment on flower and bud diameter and bud yellowing of Oncidium cut flowers after 7 days in the vase following simulated transport to Japan and methyl bromide fumigation

Simulated transport	Treatment		Floret		Diameter		7 days in vase
	TARI's oncl	MBr	on auction		5 days in vase		Bud
	+ 1-MCP	Fumi.	2 <sup>nd</sup> floret	1 <sup>st</sup> bud	2 <sup>nd</sup> floret	1 <sup>st</sup> bud	Yellowing (%)
25°C 4days	-	-	26.1±1.1	21.1 ±1.1	25.9 ±0.4	21.8 ±1.2	21.7 ±12.5
	-	+	26.3±0.9	22.4 ±0.9	26.6 ±0.8	21.5 ±1.4	32.9 ±26.0
	+	-	28.1±1.3	20.1 ±0.3	29.3 ±1.5	27.6 ±1.0	14.7 ±14.0
	+	+	29.1±1.6	21.9 ±1.8	30.0 ±1.0	26.8 ±1.4	17.1 ± 7.8
12°C 7days	-	-	28.1±2.5	20.2 ±2.4	27.3 ±3.0	22.8 ±1.6	24.8 ±17.5
	-	+	27.8±0.6	19.9 ±1.6	26.0 ±0.9	20.9 ±1.2	50.2 ±16.0
	+	-	29.2±1.3	23.4 ±2.0	29.5 ±1.7	27.6 ±1.2	12.8 ± 6.8
	+	+	29.1±1.4	24.1 ±1.7	29.7 ±1.5	27.3 ±1.2	19.8 ±11.1

Notes: 1. fumi. = fumigation for 2 hours with 24.5 g/m<sup>3</sup> of methyl bromide at 23 °C following simulated transport.

2. First bud had already opened into a flower at the time of removal from chamber.



### 3. Effect of low-pressure fumigation prior to transport on quality of Oncidium cut flowers after transport

Low-pressure fumigation with a mixture of methyl bromide and phosphine prior to transport had little effect on the auction quality of Oncidium cut flowers following simulated air and ocean transport (Table 3), but it did affect vase life and vase quality, with slight decreases in flower life span, bud life span, and bud opening rates. Bud yellowing rates also showed clear increases (Table 4).

Table 3. Effect of low-pressure fumigation with methyl bromide and phosphine on the auction quality, flower and bud senescence, vase life, flower and bud life span, and bud opening rates of Oncidium cut flowers following simulated transport.

Simulated Transport	Treatment		Auction			Vase life (day)			Bud Opening (%)
	TARI's onc 1 + 1-MCP	MBr Ph fumi.	Quality (-2→2)	Floret sene.	Bud sene.	Cut Flower	floret	Bud	
25°C 3 days	-	-	1.4 ±0.2	moderate	none	53 ±1.0	45 ±0.6	93 ±1.8	428 ±15.1
	-	+	1.3 ±0.2	moderate	none	47 ±0.4	41 ±0.4	77 ±2.7	362 ±14.9
	+	-	1.7 ±0.1	none	none	82 ±1.6	64 ±0.8	135 ±3.4	592 ±13.1
	+	+	1.5 ±0.2	slight	none	75 ±0.8	61 ±1.2	128 ±2.4	712 ±13.9
11°C 8 days	-	-	1.6 ±0.1	slight	none	50 ±1.9	43 ±0.4	120 ±2.6	465 ±12.7
	-	+	1.4 ±0.1	slight	none	44 ±0.4	36 ±0.5	90 ±3.1	310 ±10.3
	+	-	1.7 ±0.2	none	none	90 ±1.5	74 ±0.6	147 ±1.0	735 ±14.8
	+	+	1.6 ±0.1	none	none	80 ±0.6	77 ±0.4	157 ±2.9	548 ±12.2

Notes: fumi. = low-pressure fumigation at 250 torr with 10 g/m<sup>3</sup> of methyl bromide and 3 g/m<sup>3</sup> of phosphine prior to transport. Ph indicates phosphine.

2. Remaining explanations are the same as for Table 1.

Table 4. Effect of low-pressure fumigation with methyl bromide and phosphine or phosphine alone on bud yellowing of Oncidium cut flowers after 7 days in the vase following simulated transport

Simulated transport	Treatment		Bud Yellowing (%)
	TARI'S onc 1 + 1-MCP	Fumigation	
25°C 3 days	-	---	25.6 ±14.0
	+	---	7.2 ±17.5
	-	MBr+Ph	50.2 ±19.0
	+	MBr+Ph	10.0 ±12.0
	-	Ph	45.1 ±10.0
	+	Ph	14.5 ±7.0
11°C 8 days	-	---	12.7 ±11.0
	+	---	0.0 ±0.0
	-	MBr+Ph	40.6 ±18.1
	+	MBr+Ph	6.6 ±10.7
	-	Ph	36.2 ±11.9
	+	Ph	22.4 ±11.0

Note: "Fumigation" indicates fumigation prior to transport. MBr + Ph indicates fumigation with 10 g/m<sup>3</sup> of MBr + 3 g/m<sup>3</sup> of phosphine, and Ph indicates fumigation with 5 g/m<sup>3</sup> of phosphine, with this fumigation taking place for 3 hours at 250 torr in all cases.

Low-pressure fumigation with phosphine prior to transport caused a slight decrease in the auction quality of Oncidium cut flowers following simulated transport (Table 5) (see Table 3 for results in unfumigated control group), with vase life (Table 5) also showing a slight decrease, and bud yellowing rates in the vase also increased (Table 4).

4. Effect of treatment with 'TARI's Onc 1' and 1-MCP of improving quality of Oncidium cut flowers subjected to low-pressure fumigation prior to transport

Low-pressure fumigation prior to transport with methyl bromide and phosphine or with phosphine alone had a slight effect on the auction quality, vase life, and vase quality of Oncidium cut flowers following simulated transport, and treatment with 'TARI's Onc 1' and 1-MCP effectively improved all of these variables (Table 3, Table 5). Both of these types of fumigation increased bud yellowing rates in the vase, and treatment with 'TARI's Onc 1' and 1-MCP effectively reduced these rates (Table 4).

Table 5. Effect of low-pressure fumigation with phosphine on the auction quality, flower and bud senescence, vase life, flower and bud life span, and bud opening rates of Oncidium cut flowers following simulated transport

Simulated transport	Treatment		Auction			Vase life (day)			Bud Opening (%)
	TARI's onc 1 + 1-MCP	Ph fumi.	Quality (-2~+2)	Floret sens.	Bud sens.	Cut flower	Floret	Bud	
5°C 3days	-	+	1.3 ±0.2	Moderate	none	4.4 ±0.4	4.3 ±0.4	7.8 ±2.1	34.7 ±17.6
	+	+	1.5 ±0.2	Slight	none	7.7 ±0.5	7.1 ±0.5	11.3 ±1.4	58.5 ±14.4
1°C 8days	-	+	1.4 ±0.1	Moderate	none	4.8 ±0.0	4.7 ±0.3	8.8 ±2.3	30.7 ± 6.3
	+	+	1.8 ±0.2	None	none	8.2 ±0.8	7.0 ±0.6	12.7 ±2.2	40.8 ±12.0

Notes: 1. "fumi." indicates low-pressure fumigation for 3 hours at 250 torr with 5 g/m<sup>3</sup> of phosphine prior to simulated transport; "Ph" indicates phosphine.

2. Remaining explanations are the same as for Table 1.

Table 6. Effect of low-pressure fumigation with methyl bromide and phosphine or with phosphine alone on insect mortality before and following simulated transport

Time of inspection	Simulated transport	Fumigation	Mortality (%)		
			Aphis	Thrip	Worm
Before simulated transport	25°C 3days	none	18.4	14.0	---
		MBr+Ph	100.0	100.0	100.0
		Ph	88.4	98.2	100.0
	11°C 8days	none	18.9	39.4	---
		MBr+Ph	100.0	100.0	100.0
		Ph	98.3	100.0	100.0
after simulated transport	25°C 3days	none	49.0	14.8	---
		MBr+Ph	100.0	100.0	100.0
		Ph	94.7	100.0	100.0
	11°C 8days	none	46.8	19.7	---
		MBr+Ph	100.0	100.0	100.0
		Ph	98.0	99.7	100.0

Notes: 1. MBr + Ph indicates fumigation with MBr 10 g/m<sup>3</sup> + phosphine 3 g/m<sup>3</sup>, and Ph indicates fumigation with phosphine 5 g/m<sup>3</sup>, with fumigation being carried out for 3 hours at 250 torr in all cases.  
 2. *Spodoptera litura* includes 2- and 3-year cases.  
 3. --- indicates that tests were not conducted.

#### 5. Effect on insect mortality of low-pressure fumigation with methyl bromide and phosphine prior to transport

Low-pressure fumigation with a mixture of methyl bromide and phosphine caused 100% mortality of *Spodoptera litura*, *Aphis gossypii*, and *Thrips hawaiiensis* prior to and following simulated transport (Table 6). Low-pressure fumigation with phosphine alone caused 100% mortality of *Spodoptera litura* (Table 6) and virtually 100% mortality of *Thrips hawaiiensis* following transport. The effect on *Aphis gossypii* was somewhat less favorable, with survival rates after fumigation and prior to transport of 11.6% (air transport) and 1.7% (ocean transport); following simulated air and ocean transport, the survival rates were still 5.3% and 2.0% respectively (Table 6).

#### Discussion

With increasing exports of *Oncidium* cut flowers to Japan, fumigation of such flowers in Japan is also increasing, and the damage caused by such fumigation cannot be overlooked. According to exporters, the effect on *Oncidium* cut flowers of fumigation in Japan with methyl

bromide is minor, but it does reduce vase life by approximately 2-3 days (approximately 1/2 of cut flower life span). The results of this study confirmed that the effect on auction quality of the flowers was minor, but there was a decrease in vase life of 1-1.5 days (approximately 1/5 of cut flower life span), which poorly matches [illegible] exports. This may be attributable to the fact that the small numbers of flowers and careful conditions used in the present study differ from the mass transport and rough handling occurring in actual exportation, but it can be estimated that the damage caused by fumigation is much greater than the figures in the present study.

Damage to *Oncidium* cut flowers by methyl bromide fumigation involves a slow response. 1-2 days after fumigation, the cut flowers show no visible difference in quality, and the reductions in vase life and bud opening rates occur gradually thereafter. The causes of this methyl bromide-induced damage are by no means clear. In a report published by Weller and Graver (1998), damage induced by methyl bromide ( $32 \text{ g/m}^3$ ) is different in different types of cut flowers, with damage being relatively great in *Protea*, but not severe in roses and carnations. Compared to methyl bromide, phosphine fumigation can clearly reduce damage in *Protea*, and the extent of damage in roses and carnations is [illegible] therefore quite low. Treatment with  $48 \text{ g/m}^3$  was reported to cause no damage in various types of peach products (Harvey et al., 1989). 28 [illegible] after treatment of grapefruit with  $40 \text{ g/m}^3$ , the skin and pulp showed minor discoloration (Williamson et al., 1986), but none of these reports explain the cause of the action of methyl bromide. Schmidt and Christopherson (1997) reported that methyl bromide fumigation can kill thin wall tissue in oak trees and can block the action of [illegible] causing discoloration of wood, thus increasing wood quality, with the amount of methyl bromide used in this type of fumigation being as much as  $240 \text{ g/m}^3$ . As methyl bromide can destroy the [illegible], causing quite severe damage to plants, in recent years, many researchers have conducted studies in an effort to find a substitute fumigation agent (Weller and Graver, 1998, Schmidt and Christopherson, 1997) or have investigated methods for maintaining an effective insecticidal action at a lower dose of the substance (Chen et al, 1993; Zhang et al., 1994).

Treatment with 'TARI's Onc 1' and 1-MCP can increase the auction quality of *Oncidium* cut flowers fumigated with methyl bromide following transport, making their quality as high as that of unfumigated flowers, and this type of treatment can also restore vase life and quality to the same or better levels than in unfumigated flowers, with the result that *Oncidium* cut flowers exported to Japan can still retain favorable quality after fumigation. Comparing flowers treated with 'TARI's Onc 1' and 1-MCP and unfumigated flowers, there is a [illegible], but fumigated

flowers are still somewhat inferior, and prevention of fumigation in Japan therefore remains the best approach.

Disinfestation prior to transport can reduce fumigation in Japan, and in addition to the fact that relatively little treatment is required, cut flower freshness remains high, damage incurred is fairly small, the flowers can clear customs on time and be auctioned on time, and their price can be controlled. In this study, we investigated the effect of low-pressure fumigation with a low dose of methyl bromide mixed with phosphine on the auction quality and vase life of *Oncidium* cut flowers following simulated transport, and found that at a low fumigant dose compared to that used in Japan, this method effectively eliminated three important insect pests, i.e., *Spodoptera litura*, *Aphis gossypii*, and *Thrips hawaiiensis* (inducing 100% mortality). We therefore feel that further research on practical applications of this method is warranted. The effect on insect mortality of phosphine alone is also fairly good, but although this fumigant makes the bud opening rate in the vase fairly high, its effect on the overall quality of the flower is relatively minor, and its damaging effect on product quality can be alleviated to a significant degree by treatment with a mixture of 'TARI's Onc 1' and 1-MCP. Use of a combination of these two methods will make it possible to carry out sufficient disinfestation of flowers before they are imported so as to prevent the drawbacks of fumigation in Japan, and simultaneously keep product quality high after export. It was therefore concluded that it will be worthwhile to conduct extensive research on this method.

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